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Effect of intra-abdominal cisplatin on post-laparotomy adhesion bands in rats

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Abstract

Background: Intra-abdominal adhesion is one of the major problem of postoperative surgery. It causes abdominal, and gynaecological morbidity. Postoperative intraperitoneal chemotherapy (IPCT) has been proved to be effective in patients with advanced cancer after cytoreductive surgery compared to systemic chemotherapy. In recent years there are several studies have been conducted to evaluate the role of IPCT on cancer treatment wound healing, and adhesion formation.
Objectives: The aim of this experimental study was to evaluate the effect of intra-abdominal cisplatin on postoperative adhesion formation during 2nd and 4th weeks after surgery.
Method: For conducting this experimental study, 40 male Wistar rats were studied and divided into two groups of twenty animals each. The abdomen was prepared for laparotomy. They underwent a laparotomy through a 3 cm midline incision. Then five abrasions as 3-4 cm were made on cecum the peritoneal wall. Control and treatment groups were received saline 0.9% (2 ml) and cisplatin (2 mg/kg) respectively during laparotomy intra-abdominally. Adhesion grade score was observed 2nd and 4th weeks after laparotomy.
Results: As the results, 10% of animals in the cisplatin groups have zero adhesion score compared to control group (11%) 4th week after surgery that this difference was not significant (P=0.964). The control group with score 3 (44.4%), and cisplatin group with score 1 (55.6%) had most adhesion in the 2nd week. Most adhesion were happened with score of 1 and 3 for both control (33.3%) and cisplatin groups (40%) respectively, 4th week after surgery (P=0.964). Moreover, there was no significant difference between two groups as the adhesion score (0-1) and (2-3) in the 2nd and 4th weeks after surgery (P=0.75).
Conclusion: Administration of intra-abdominal cisplatin in rats during laparotomy could induce adhesion band similar to control group. It seems that notice to chemotherapeutic agents, range of doses, number of pulse therapy, time of administration after operation, improving their formulation, and solvents application are necessary.

Keywords: Intra-abdominal, Laparotomy, Cisplatin, Adhesion, Rats

1. Background

Intra-abdominal adhesions following surgery as a major unsolved problem, could happen after 50% to 100% of all surgical interventions in the abdomen and cause remarkable complicates (1). Pelvic and abdominal adhee sions have been reported with significant gynaecological morbidity, such as infertility, chronic pelvic pain, small bowel obstruction, and difficulty with surgical complications (2).

There are some mechanical barriers for adhesion prevention that has been approved by Food and Drug Administration including hyaluronic acid/carboxymethylcellulose (Seprafil), oxidized regenerated cellulose (Interceed), and expanded polytetrafluoroethylene (Gore-Tex) (3). It seem that gels and hydroflotating, as an effective adhesion prevention agents, can be useful during gynaecological surgery. But, there is no evidence to show any improvement fertility outcome or pelvic pain(4). However, it has reported that there is no evidence of the effects of these barriers on pain or fertility outcomes in the women of reproductive age. There is few evidence about the efficacy of Interceed, Gore-Tex, and Seprafilm in reducing the incidence of adhesion formation following pelvic surgery (5). It has been shown that hyaluronic acid/carboxymethyl membrane (HA/CMC) could reduce incidence, extent and severity of adhesion. However, the incidence of intestinal obstruction was not decreased (6).

Intraperitoneal chemotherapy (IPCT) developing as a new oncological treatment has been used for locoregional control and long-term survival (7, 8). One of the important problem in the patient with colonic cancer

is the early postoperative adhesion formation and these adhesions could decrease the effect of IPCT. They may inhibit the distribution of the antineoplastic agents (7, 9). In study by Khoe et al., 2009, it was shown that from the 307 eligible patients that have received at least one dose of IPCT, 34% of them had developed symptomatic intestinal obstruction after IP therapy. However, the incidence of adhesion bowel obstruction was 4%. Then, the majority of the obstructions were related to the progression of malignant intra-abdominal disease (10). On the other hand, one meta-analysis has shown that IPCT could increase overall survival and progression-free survival from advanced ovarian cancer (11).

2. Objectives

Thus, the aim of this study was to investigate the effects of intra-abdominal cisplatin on adhesion band formation after laparotomy in rats.

3. Material and methods

3.1. Animals

Forty male Wistar rats (200–220 g) were provided from the Razi Institute (Karaj, Iran. Animals were kept at constant room temperature (21±2 °C) under a normal 12 Light:12 Dark cycle with free access to food and water. All animal experiments were carried out in accordance with the European Communities Council directive of 24 November 1986 (86/609/EEC) in such a way as to minimize the number of animals and their suffering.

3.2. Surgery and experimental procedures

For this study, 40 male Wistar rats were used and divided into two groups of twenty animals each (n=20). Rats were anesthetized with ketamine (60 mg/kg i.p.) and xylazine (6 mg/kg i.p.) and). The abdomen was shaved and prepared with povidone-iodine. For laparotomy, all animals underwent a laparotomy through a 3 cm midline incision and the cecum was identified and the surgeon held it gently between thumb and index fingers. Then five abrasions as 3-4 cm were made on cecum. Also the same abrasions in number and lengths were made on the peritoneal wall (12). Then, control and treatment groups were administrated saline 0.9% (2 ml) and cisplatin (2 mg/kg) respectively during laparotomy intra-abdominally. Finally, the cecum was placed back in the abdomen and the abdomen was closed in 2 layers using nylon string and running sutures. The rats then were followed daily for wound healing, and general status to assure no delayed postoperative complications. After 2nd and 4th weeks of laparotomy, half of the animals in each groups were transferred to the experimental room and animals were anesthetized and subjected to laparotomy through the previous midline incision. Adhesion grade score was given by a blind observer as follow: 0 no adhesions, 1 filmy adhesion and blunt dissection, 2: strong adhesion and sharp dissection, 3 very strong vascularized adhesions, sharp dissection (13).

3.3. Statistical analysis

Statistics were calculated using SPSS software. Results are expressed as the percent of observed adhesion as the adhesion grade score in each group. Statistical comparison between groups was estimated using with chi-square test. A level of $P < 0.05$ were considered as statistically significant.

4. Results

Two animals of control were died after surgery on days 1 and 3. Also, and one animals in the cisplatin group died on day 7. Our results showed that 11% and 10% of animals in the cisplatin groups have zero adhesion score compared to control group (11%) 2nd and 4th weeks after surgery that this difference was not significant (P=0.964).

The control group with score 3 (44.4%), and cisplatin group with score 1 (55.6%) had most adhesion in the 2nd week. However, in the 4th week, most adhesion were happened with score of 1 and 3 for both control (33.3%) and cisplatin groups (40%) respectively that this difference was not significant (P=0.964) (Table 1).

Furthermore, there was no significant difference between two groups as the adhesion score (0-1) and (2-3) in the 2nd and 4th weeks after surgery (P=0.75). However, it seems that adhesion score (2-3) of cisplatin group in the 2nd week was lower than control. But, it was not significant (Table 2).

Post-operative adhesion formation in the control and cisplatin groups with adhesion grade scores at different times was shown in Fig 1-2.

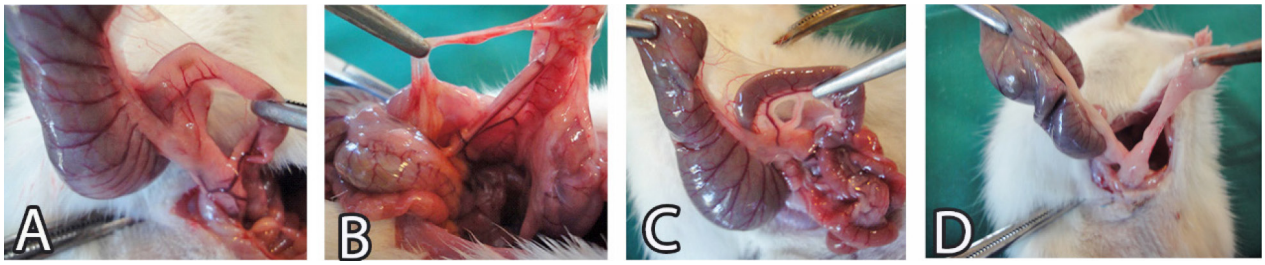
Table 1. The comparison between the peritoneal adhesion grade score between two groups of control and cisplatin on 2nd and 4th weeks post-operation in rats

Adhesion grade score	Control 4th w, n(%)	Control 2nd w, n (%)	Cisplatin 4th,w, n (%)	Cisplatin 2nd w, n (%)	P value
0	1(11%)	1(11%)	1(10%)	1(11%)	
1	3(33.3%)	3(33.3%)	4(40%)	5(55.6%)	0.964
2	2(22.2%)	1(11%)	1(10%)	0(0)	
3	3(33.3%)	4(44.4%)	4(40%)	3(33.3%)	

Table 2. The comparison between two groups of control and cisplatin as the adhesion score (0-1), and (2-3) on 2nd and 4th weeks post-operation in rats

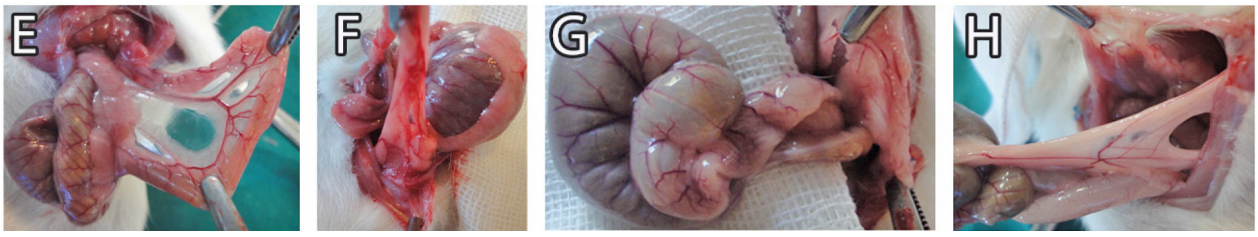
Adhesion grade score	Control 4th w, n(%)	Control 2nd w, n (%)	Cisplatin 4th w, n (%)	Cisplatin 2nd w, n (%)	P value
Score (0-1)	4(44.4%)	4(44.4%)	5(50.0%)	6(66.7%)	
Score (2-3)	5(55.6%)	5(55.6%)	5(50.0%)	3(33.3%)	0.75

Fig 1. Comparison of post-operative adhesion formation between control and cisplatin groups on the 2nd week



A: Control, score 2; B: Control, score 3; C: Treatment, score 2, D: Treatment, score 3

Fig 2. Comparison of post-operative adhesion formation between the control and cisplatin groups on the 4th week



E: Control, score 1, F: Control, score 3, G: Treatment, score 1, H: Treatment, score 3

5. Discussion

Our result showed that intraperitoneal adhesion induced by laparotomy between the two groups of saline and cisplatin, was not significant on the 2nd and 4thweeks after surgery in rats. In women with ovarian, tubal, or peritoneal malignancies, the rate of bowel obstruction due to intra-abdominal adhesions was reported 4% of the total number of patients after IPCT. Among the all type of chemotherapy agents, cisplatin has been administrated IP for patients and a fibrous coating bowel was seen in some of them (10). In another study, IP administration of cisplatin for treating intra-abdominal tumor has induced 6% abdominal adhesion (7 of 115 patients). Interesting issue in this study is that chemotherapy cycles were not happened during post-operation period. Thus, it was suggested that adhesion formation was related to the local toxicity of cisplatin not traumatic effect of surgical wound (14, 15). Similarly, our study has shown that administration of cisplatin during laparotomy induced adhesion score compared to control group that has received saline. However this difference was not significant compared to control. It seems that animal results could confirm clinical results two above clinical results.

In a study by Conroy et al., 2003, it has reported that delivery solution for injection, the administration way of drug for example injection such as pump or bolus could be important in adhesion formation. Phosphate-buffered saline (PBS) and Ringers' lactate solution (RLS) as delivery solutions for IP administration of doxorubicin (Dox) and bleomycin respectively could not prevent adhesion formation. But, icodextren as a delivery solution for chemotherapeutic drugs compared to both of them could significantly reduce adhesion formation. Furthermore, it seems that administration of single injection of lowest dose of bleomycin in a tumor-free animal was not effective in adhesion formation (16). Unlike to above study, we have used saline as a solution and single dose of intra-abdominal cisplatin (2 mg/kg) could induce peritoneal adhesion. This may be related to irritant properties of chemotherapeutic agents that we have mentioned it before (15).

On the other hand, there are some studies about the effective role of chemotherapy on the prevention of adhesion formation (7, 9). Thus, it seem that type of chemom therapy agent in the induction or prevention of adhesion is effective.

Furthermore, it has shown that application of high dose of Dox-loaded poly (ethylene glycol)–poly (ε-caprolactone)–poly (ethylene glycol) (PECE) micelles (Dox-M) (5 mg/kg) in the abdominal cavity of mice could show both antitumor and antiadhesion effects. About the mechanism of drug, it seems that in the early stage after administration of Dox-M, it inhibits re-mesothelialization of the injured sites and the activity of fibroblasts due to its high concentration. But, after degradation of the micelles and gradually decreasing the concentration of Dox, injured part of peritoneum starts to re-mesothelialize. The role of micelles in preventing adhesion is unclear. But, it seems that micelles by preventing the invasion of fibroblast, provide suitable condition for regeneration of mesothelial. Another possibility is that micelles by sticking to the peritoneal wounds could prevent fibrinous adhesion between the adjacent peritoneal wounds (17). As the above study, it seems that the kind of formulation and uses biodegradable micelles for chemotherapy agent could be important in the prevention of adhesion formation or severity of adhesion.

In a case of 71-year-old man, with obstructive descending colon cancer, and massive peritoneal metastases which has received palliative surgery, bowel strangulation was happened on day 4 of the 2nd course of chemotherapy, which contains irinotecan, l-leucovorin, and 5-fluorouracil (5-FU). Massive intraperitoneal adhesion was formed around several intestinal loops that was not observed in previous surgery. It seems that in parallel with the severe peritoneal metastases, inflammatory reactions induced by chemotherapy throughout the abdomen cause the formation of massive peritoneal adhesion. It was suggested that in patients with multiple peritoneal metastases, effective chemotherapy will cause peritoneal adhesion that should be considered (18).

Contrary to above study, multiple cycles of IP 5-FU have not been associated with abdominal adhesion. However, numerous cycles of IP mitomycin C are not well tolerated in patients, and appear to cause a long term adhesive process in the abdominal cavity. Furthermore, the incidence of reoperations for intra-abdominal adhesions in patients who receives one more cycle of IP mitomyin C was significantly higher than 5-FU (19). As above studies, it seems that not only chemotherapy agents, schedule, and systemic or IP administrative way of drug, but also severity of peritoneal metastasis is in formation of adhesion is effective.

The aim of this study was to investigate the effects of intra-abdominal administration of cisplatin on adhesion formation on post laparotomy rats and we have observed that cisplatin has no effect on post-laparotomy abdominal adhesion compared to the control group in the different times. However, limitation of our study may be related to short time of study, single dose of cisplatin, and applying saline as a solvent.

5.1. Conclusion

Our study showed that administration of intra-abdominal cisplatin in rats undergoing laparotomy has no effect on post-laparotomy abdominal adhesion compared to the control group. It seems that attention to type of chemotherapeutic agents, range of doses, number of pulse therapy, improving their formulation, and solvents application are necessary. This study could pave the road to develop new interventional clinical studies for preventing or reducing adhesion in patients who receiving different doses of chemotherapeutic agents.

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Footnote

Authors' Contributions: Mohmmadreza Amirbaigloo was investigator of the study. Zahra Farahani-Nick has designed and supervised the study. Marjan Nassiri-Asl was involved in the interpretation of results, wrote the paper, and revised it. Zoreh Yazdi has analyzed the data.

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